either independently or simultaneously in the same screen, with the donor variable region will identify those binders that have substantially the same or greater binding affinity as the donor. Those skilled in the art will know, or can determine using the donor variable region, binding conditions which are sufficient to identify selective interactions over non-specific binding.

Detection methods for identification of binding species within the population of altered variable regions can be direct or indirect and can include, for example, the measurement of light emission, radioisotopes, colorimetric dyes and fluorochromes. Direct detection includes methods that operate without intermediates or secondary measuring procedures to assess the amount of bound antigen or ligand. Such methods generally employ ligands that are themselves labeled by, for example, radioactive, light emitting or fluorescent moieties. In contrast, indirect detection includes methods that operate through an intermediate or secondary measuring procedure. These methods generally employ molecules that specifically react with the antigen or ligand and can themselves be directly labeled or detected by a secondary reagent. For example, a antibody specific for a ligand can be detected using a secondary antibody capable of interacting with the first antibody specific for the ligand, again using the detection methods described above for direct detection. Indirect methods can additionally employ detection by enzymatic labels. Moreover, for the specific example of screening for catalytic antibodies, the disappearance of a substrate or the appearance of a product can be used as an indirect measure of binding affinity or catalytic activity.

Isolated variable regions exhibit binding affinity as single chains, in the absence of assembly into a heteromeric structure with their respective V_H or V_L subunits. As such, populations of V_H and V_L altered variable regions polypeptides can be expressed alone and screened for binding affinity having substantially the same or greater binding affinity compared to the CDR donor V_H or V_L variable region. Alternatively, populations of V_H and V_L altered variable regions polypeptides can be coexpressed so that they self-assemble into heteromeric altered variable region binding fragments. The heteromeric binding fragment population can then be screened for species exhibiting binding affinity substantially the same or greater than the CDR donor variable region binding fragment. A specific example of the coexpression and self-assembly of populations V_H and V_L altered variable regions into hetermeric populations is described further below in the Examples.

Therefore, the invention provides a method of simultaneously grafting and optimizing the binding affinity of a variable region binding fragment. The method consists of: (a) constructing a population of altered heavy chain variable region encoding nucleic acids consisting of an acceptor variable region framework, containing donor CDRs and a plurality of different amino acids at one or more framework region and CDR amino acid positions; (b) coexpressing the populations of heavy and light chain variable region encoding nucleic acids to produce diverse combinations of heteromeric variable region binding fragments, and (c) identifying one or more heteromeric variable region binding fragments having affinity substantially the same

or greater than the donor CDR heteromeric variable region binding fragment.

The invention additionally provides a method of optimizing the binding affinity of an antibody variable region. The consists of: (a) constructing a population of antibody variable region encoding nucleic acids, said population comprising two or more CDRs containing a plurality of different amino acids at one or more CDR amino acid positions; (b) expressing said population of variable region encoding nucleic acids, and (c) identifying one or more variable regions having binding affinity substantially the same or greater than the donor CDR variable region.

The methods described above, for conferring donor CDR binding affinity onto an antibody acceptor variable region framework and for simultaneously grafting and optimizing the binding affinity of a heteromeric variable region binding fragment, can additionally be employed to modify or optimize the binding affinity of a variable region or a heteromeric variable region binding fragment. Similar to the previously described methods, the method for modifying or optimizing binding affinity involves the selection of relevant amino acid positions and the construction, expression and screening of variable region populations containing variable amino acid residues at all or a fraction of the selected positions. However, for optimization of binding affinity it is not necessary to vary amino acid positions in the framework regions. Instead, all that is required is to alter one or more amino acid positions in two or more CDR regions. Changing the CDR amino acid residues directly effects the binding affinity. Once a population containing variable amino acid residues incorporated in